Hemochromatosis Gene Mutations and Distal Adenomatous Colorectal Polyps

Katherine A. McGlynn,[1] Lori C. Sakoda,[1] Ying Hu,[2] Robert E. Schoen,[4] Robert S. Bresalier,[5] Meredith Yeager,[3] Stephen Chanock,[1,2,3] Richard B. Hayes,[1] and Kenneth H. Buetow[2]

[1]Division of Cancer Epidemiology and Genetics, [2]Center for Cancer Research, and [3]Core Genotyping Facility, National Cancer Institute, NIH, Department of Health and Human Services; [4]University of Pittsburgh, Pittsburgh, Pennsylvania; and [5]M.D. Anderson Cancer Center, Houston, Texas

Abstract

Iron has been suggested to be a risk factor for colorectal neoplasia. Some individuals who are heterozygous for mutations in the hemochromatosis gene (*HFE*) have higher than average serologic measures of iron. We therefore investigated whether heterozygosity for *HFE* mutations was related to risk of advanced distal adenoma and whether the relationship was affected by dietary iron intake. In the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, 679 persons with advanced distal adenoma and 697 control persons were genotyped for the two major *HFE* mutations (C282Y and H63D), one *HFE* polymorphism (IVS2+4), and one polymorphism (G142S) in the transferrin receptor gene (*TFRC*). *HFE* haplotypes were also created to examine the effect of haplotype on risk. Food frequency questionnaire data were used to estimate daily iron intake. There was no relationship between any *HFE* genotype or haplotype and advanced adenoma. Stratification of *HFE* genotype by *TFRC* genotype did not change the results. In addition, there was no relationship between dietary iron intake and risk of adenoma or between *HFE* genotype and risk of adenoma, stratified by iron intake. These results do not support a relationship between *HFE* heterozygosity and risk of advanced distal adenoma. (Cancer Epidemiol Biomarkers Prev 2005;14(1):158–63)

Introduction

Both iron intake and serologic measures of body iron have been reported to increase the risk of colorectal cancer (1, 2) and precancerous colonic adenoma (3, 4). The biological mechanism behind the association is thought to be related to the ability of iron to catalyze the formation of free radicals (5, 6). Free radicals can cause damage to lipids, proteins, and DNA of colonic cells and can mediate the activation of procarcinogens to carcinogens (7). In addition, iron has been shown to act as an immune suppressant and to promote the growth of cancer cells (8, 9).

Hereditary hemochromatosis (HH) is a disease characterized by excessive iron absorption from the gut (10). Geneticallly, HH is an autosomal recessive disease. Positional cloning studies have identified the hemochromatosis gene (*HFE*) on chromosome 6 as the major determinant of HH (11). Two functionally significant missense mutations in the *HFE* gene have been identified The major mutation, a GA substitution at nucleotide 845 in exon 4, results in the replacement of a cysteine by a tyrosine at amino acid 282 (C282Y). The C282Y mutation is thought to be of either Scandinavian or Celtic origin and is most common among persons of northern European ancestry (12). The second *HFE* mutation, a CG replacement at nucleotide 187 in exon 2, results in the replacement of a histidine by an aspartic acid at amino acid 63 (H63D). The H63D mutation is thought to be older than the C282Y mutation and is more common among persons of southern European ancestry (12). Neither the C282Y nor the H63D mutation is common among persons of Asian and African ancestries. In addition to the two major mutations, several other *HFE* polymorphisms have been identified (13).

Studies of patients with HH of European ancestry have reported considerable variability in the frequency of C282Y mutation homozygotes, with a mean frequency of 84% and a range of 60% to 100%, depending on geographic location (14). On average, 4% of patients with HH of European ancestry are homozygous for the H63D mutation, 6% are compound heterozygotes for the C282Y and H63D mutations, and ~8% carry neither mutation (14).

Studies of serum iron indices in persons with hemochromatosis have reported significantly increased ferritin and transferrin saturation levels. Studies of persons heterozygous for *HFE* mutations (genetic heterozygotes) and parents of hemochromatosis patients (obligate heterozygotes) have reported somewhat higher ferritin and transferrin saturation values as well, but not as high as persons with hemochromatosis.

Whether heterozygotes are at increased risk of colorectal neoplasia is unclear. Several studies have reported an increased risk among heterozygotes (3, 15, 16). One of the studies reported that the risk was increased only in combination with the G142S polymorphism in the transferrin receptor 1 gene (*TFRC*; ref. 15). These positive results, however, have not been replicated in other studies (17-21). We therefore sought to examine the question in a large, well-characterized screening study, the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Because iron intake may be a risk factor for colorectal neoplasia and may affect *HFE* gene penetrance, we also examined the relationship between *HFE* mutations and colorectal neoplasia by levels of iron intake.

Materials and Methods

The study was conducted using a nested case-control design within the PLCO Cancer Screening Trial (22). The trial recruited 154,952 participants, ages 55 to 74 years, at 10 U.S. study centers (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St. Louis, MO; and Washington, DC). Participants who were randomly assigned to the screening arm of the PLCO

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Requests for reprints: Katherine A. McGlynn, Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, EPS-7060, 6120 Executive Boulevard, Rockville, MD 20852-7234. Phone: 301-435-4918; Fax: 301-402-0916. E-mail: mcglynnk@mail.nih.gov

trial were offered a sigmoidoscopy examination at study entry. At sigmoidoscopy, the examiner recorded the location and shape and estimated the size of each of the four largest lesions. Lesions were usually not biopsied or removed. Individuals were referred to their personal physicians for evaluation of screen-detected abnormalities and were tracked to determine the results from subsequent diagnostic workup. Data on diagnostic follow-up with repeat flexible sigmoidoscopy or colonoscopy were collected by trained medical record abstractors, who recorded the pathology, size, and location of each lesion found. Written informed consent was obtained from participants and the trial received approval from the institutional review boards of the U.S. National Cancer Institute and the 10 study centers.

Study Population. Study participants were selected from among 42,037 individuals in the screening arm of the trial who underwent a successful sigmoidoscopic examination (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified) between September 1993 and September 1999. At entry, participants completed risk factor and dietary questionnaires and donated blood for use in etiologic studies. After exclusion of 4,834 individuals with a self-reported history of cancer (except basal cell skin cancer), ulcerative colitis, Crohn's disease, familial polyposis, colorectal adenoma or Gardner's syndrome, we randomly selected 720 of the 1,234 cases with advanced distal adenoma (adenoma ≥1 cm or containing high-grade dysplasia or villous elements). Advanced adenoma were preferentially selected because their likelihood of becoming malignant is greater than that of less advanced adenoma. An equal number of gender- and ethnicity-matched individuals (n = 733) with a negative screening sigmoidoscopy (i.e., no polyp or other suspect lesion, n = 26,651) were selected as controls. Genotype and genetic fingerprint analyses were attempted on samples from all selected participants and were successfully obtained from 679 cases and 697 controls.

Genotype Analysis. Genotying of the two *HFE* mutations (C282Y and H63D) and the *TFRC* G142S polymorphism was accomplished utilizing 5′ nuclease assays (TaqMan). Also using TaqMan assays, a polymorphism in intron 2 of *HFE* (IVS2+4; ref. 23) was typed to facilitate the construction of *HFE* haplotypes. Briefly, 10 ng of lyophilized sample DNA was used for each 5-L TaqMan reaction. The reactions were done in a 384 (96 × 4)-well plate format. In addition to the study samples, four Coriell DNA controls for each genotype and no-template controls were put on each plate; 2.5 L of the 2× Universal Mater Mix (ABI, Foster City, CA) and assay-specific concentrations of primers and probes were used in the reaction. The following assay-specific thermocycling conditions were used: step 1, 50°C for 2 minutes (AmpErase UNG activation, ABI); step 2, 95°C for 10 minutes (enzyme activation); step 3, 92°C (if using 3′ MGB quencher) or 95°C (if using 3′ TAMRA quencher) for 30 seconds (template denaturation); step 4, 60°C for 1 minute (assay-specific annealing); step 5, repeat step 3, 49 times; step 6, hold at 4°C. The plates are then read on the ABI 7900HT sequence detection system. All primers and probes can be viewed at the following Web site: http://snp500cancer.nci.nih.gov/snp.cfm by entering the appropriate SNP IDs: HFE-01 is the ID of *HFE* H63D, HFE-02 is the ID of *HFE* C282Y, HFE-03 is the ID of *HFE* IVS2+4, and TFRC-01 is the ID of *TFRC* G142S.

Each sample was also genetic fingerprinted using the AmpFLSTR Profiler Plus panel (Applied Biosystems, Foster City, CA). The panel includes nine tetranucleotide single tandem repeat loci (D31358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, vWA) as well as the sex-specific Amelogenin locus. Genetic fingerprint analysis provided the opportunity to assess potential population stratification.

Assessment of Questionnaire-Based Factors. At the initial screening visit, participants were asked to complete a questionnaire about sociodemographic factors, medical history, and risk factors for cancer. Usual dietary intake over the 12 months before enrollment was assessed with a 137-item food frequency questionnaire including an additional 14 questions about intake of vitamin and mineral supplements (PLCO). Daily dietary nutrient intake was calculated by multiplying the daily frequency of each consumed food item by the nutrient value of the gender-specific portion size (24) using the nutrient database from the U.S. Department of Agriculture (25). Total iron intake was calculated by adding dietary and supplemental iron intakes.

Statistical Analysis. *HFE* and *TFRC* genotype frequencies among the controls were compared with the frequencies expected by Hardy-Weinberg equilibrium using goodness-of-fit χ^2 tests. Before constructing *HFE* haplotypes, linkage disequilibrium analysis was conducted to confirm the presence of linkage disequilibrium among the loci (26). Linkage disequilibrium parameters (D and D') and the χ^2 tests for statistical significance and their corresponding P values were calculated.

The *HFE* haplotypes were constructed using the SNPHAP software http://www-gene.cimr.com.ac.uk/clayton/software/stata). SNPHAP uses an expectation maximization algorithm to calculate maximum likelihood estimates of haplotype frequencies given genotype measurements that do not specify phase (27). Estimation was not used to construct haplotypes for individuals lacking genotype data at any of the three *HFE* loci. Only four haplotypes occurred in the study population, consistent with family data that have shown that the C282Y and H63D mutations do not occur on the same chromosome and the H63D mutation is always paired with the IVS2+4 variant allele. The resulting four haplotypes of (H63D-IVS2+4-C282Y) are (*a*) wild-type-wild-type-wild-type, (*b*) wild-type-variant-wild-type, (*c*) wild-type-wild-type-mutant, and (*d*) mutant-variant-wild-type.

Using unconditional logistic regression analysis, prevalence odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for the *HFE* and *TFRC* genotypes and for the *HFE* haplotypes as a measure of association with case-control status. Estimates for colon adenoma risk were also computed for genotype combinations of variants at the three *HFE* loci by *TFRC* genotype status. We adjusted all ORs for sex, age at randomization (55-59, 60-64, 65-69, 70-74), smoking history (never, former, current), family history of colorectal cancer (no, yes), total iron intake, total fiber intake, and total energy intake. Dietary nutrients were categorized into quartiles with cutpoints determined according to their distributions among the controls. Other potential risk factors for colorectal tumors, including educational attainment, aspirin use, ibuprofen use, physical activity, body mass index, red meat intake, and folate intake did not appreciably change the risk estimates and were not included in the final analytic models. In addition, we assessed whether the relationship between any *HFE* mutation and risk of colon adenoma was modified by increasing age or by higher iron intake levels.

Logistic regression analyses were conducted using SAS version 8.2 (SAS Institute, Cary, NC). Population stratification analysis was conducted using STRUCTURE (available at http://pritch.bsd.uchicago.edu/software/readme_2_1/readme.html; ref. 28). EHplus, available on the Medical Research Council Rosalind Franklin Centre for Genomics Research Web site at http://www.hgmp.mrc.ac.uk, was used to conduct linkage disequilibrium analysis (29).

Results

The population identified for the study included 720 individuals with advanced adenoma and 733 controls. Given the limited sample size, non-White individuals (44 cases, 47

Table 1. Distribution of covariates among cases and controls

Variable	Cases (n = 635), n (%)	Controls (n = 650), n (%)	P (χ^2 test)
Sex*			
Male	443 (69.8)	449 (69.1)	0.79
Female	192 (30.2)	201 (30.9)	
Age (y)			
55-59	208 (32.8)	299 (46.0)	
60-64	200 (31.5)	172 (26.5)	
65-69	143 (22.5)	118 (18.2)	<0.0001
70-74	84 (13.2)	61 (9.4)	
Smoking status			
Never	243 (38.3)	302 (46.5)	
Former	309 (48.6)	309 (47.5)	<0.0001
Current	83 (13.1)	39 (6.0)	
NSAID use†			
Yes	368 (58.0)	390 (60.0)	0.46
No	267 (42.0)	260 (40.0)	
Family history of colorectal cancer‡			
Yes	77 (12.1)	60 (9.2)	0.09
No	558 (87.9)	590 (90.8)	
Total iron intake (mg/d)§			
Q1 (0-16.58)	190 (29.9)	162 (24.9)	
Q2 (16.59-24.88)	139 (21.9)	163 (25.1)	
Q3 (24.89-35.36)	172 (27.1)	162 24.9	0.07
Q4 (≥35.37)	134 (21.1)	163 (25.1)	
Total fiber intake (g/d)§			
Q1 (0-17.01)	189 (29.8)	162 (24.9)	
Q2 (17.02-22.73)	164 (25.8)	163 (25.1)	
Q3 (22.74-29.10)	146 (23.0)	162 (24.9)	0.16
Q4 (≥29.11)	136 (21.4)	163 (25.1)	
Total energy intake (kcal/d)§			
Q1 (0-1,631.71)	195 (30.7)	162 (24.9)	
Q2 (1,631.72-2,067.16)	136 (21.4)	163 (25.1)	
Q3 (,2067.17-2,627.21)	160 (25.2)	162 (24.9)	0.09
Q4 (≥2,627.22)	144 (22.7)	163 (25.1)	

NOTE: Abbreviation: NSAID, nonsteroidal anti-inflammatory drug.
*Controls were matched to cases on sex.
† NSAID use = any use of aspirin or ibuprofen.
‡ Family history = family history in first-degree relatives.
§Quartiles were determined using the control data.

controls) were not included in the final analysis. Individuals with incomplete genotype information (24 cases, 22 controls) or genetic fingerprint data (17 cases, 14 controls) were not included as we desired to create haplotypes for all participants and to examine population stratification. Thus, a total of 1,285 individuals (635 cases and 650 controls) were included in the final data analysis, ~70% of whom were male (Table 1). The cases were significantly older than the controls (P < 0.0001) and more likely to be current or former smokers (P < 0.0001). There was no difference between the cases and controls in total energy intake, family history of colorectal cancer, or nonsteroidal anti-inflammatory drug use. In addition, there was no significant difference in total iron intake (P = 0.07), although the controls tended to have a somewhat higher iron intake than the cases. Although there was no difference between the cases and controls in fiber intake when fiber was categorized into quartiles, there was a significant difference when quartiles 1 and 2 were contrasted with quartiles 3 and 4 (P = 0.045), with cases having lower fiber intake than controls.

Genetic fingerprint analysis determined that significant population stratification did not exist (data not shown). As a result, the medical center was not included as a variable in the analysis.

Among the controls, the genotype distributions of all four loci examined were in agreement with Hardy-Weinberg equilibrium (Table 2). The allele frequencies of the C282Y and H63D mutations were 6.3% and 13.2%, respectively. Both observed frequencies were consistent with reported frequencies of 6.2% and 15.1% in the U.S. White National Health and Nutrition Examination Survey (NHANES) III population (30) and of 6.2% and 15.2% in the White population of a large California health care maintenance organization (31).

Linkage disequilibrium analysis confirmed significant linkage disequilibrium existed among the three *HFE* mutations/polymorphisms. For the case participants, χ^2 = 372.7, P < 0.0001, and for the control participants, χ^2 = 332.3, P < 0.0001. The pairwise D' values ranged between 0.995 and 0.999.

The genotype frequencies of the four loci among cases and controls are shown in Table 2. There was no significant difference in any of the genotype distributions between the cases and controls, whether estimated by crude ORs or by ORs adjusted for sex, age, smoking, family history, and intakes of total iron, total fiber, and total energy. Analysis of trends found that there were no significant trends in any of the genotypes. Similarly, when *HFE* genotypes were combined, as shown in Table 3, there were no significant differences in crude or adjusted ORs between cases and controls. The distributions of *HFE* genotype combinations, stratified by *TFRC* G142S genotype are also shown in Table 3. Stratification by *TFRC* had little effect on the ORs in either stratum. In particular, individuals who had a combination of the *TFRC* G142S AA genotype and any *HFE* mutation were at no greater risk of colorectal neoplasia than were other individuals, as had been previously reported (15). One comparison (persons with wild-type *HFE* genotypes and a *TFRC* G142S genotype of either GG or GA) did attain formal statistical significance, but did not remain significant after adjustment for multiple comparisons.

Table 4 presents the results of *HFE* haplotype analyses. Haplotypes are presented in the genomic order in which they appear on the *HFE* gene, H63D-IVS2+4-C282Y. W represents the wild-type allele, and C (cytosine), A (adenine), and G (guanine) represent the mutant alleles (for H63D and C282Y) or the variant allele (for IVS2+4). None of the four haplotypes were significantly related to case status.

Discussion

We examined the relationship of *HFE* mutations with advanced adenoma in a nested case-control study. We found no evidence that either of the major hemochromatosis mutations was related to adenoma. In addition, we found no evidence that the *HFE* mutations or the *HFE* IVS2+4 polymorphism were related to adenoma when stratified on *TFRC* G142S genotype. Similarly, we noted no significant associations between *HFE* haplotypes and colonic adenoma.

Whether there is a relationship between *HFE* mutations and colorectal neoplasia has been the subject of some debate in the literature. At least three studies, two of which are based on genotypes, have reported a significant association (3, 15, 16), whereas five studies, three of which were based on genotypes, in addition to the present one, have not found an association (17-21).

Nelson et al. (3) reported that parents of patients with HH were significantly more likely to have a history of colonic adenoma [relative risk (RR), 1.29; 95% CI, 1.08-1.53 in mothers; RR, 1.24; 95% CI, 1.07-1.53 in fathers] than were parents of the spouses of patients with HH. In addition, fathers of the patients with HH were more likely to have had a history of colorectal carcinoma (RR, 1.28; 95% CI, 1.07-1.53). Although the study was large, including information on 1,950 parents of patients with HH and 1,656 parents of patients without HH, the response rate was low (44%), the parents' medical histories were obtained by questionnaire from their children and the medical histories were not verified. In addition, neither the patients nor their parents were genotyped, although it is likely that most parents were heterozygotes.

In a Swedish case-control study of colorectal cancer, Beckman et al. (15) reported that there was no significant difference between cases and controls in the genotypic distributions of either the *HFE* C282Y mutation or the H63D mutation. When the data were stratified by *TFRC* G142S genotype, however, the investigators found an increased risk of colorectal cancer among individuals with one or more C282Y mutant alleles who also had a *TFRC* AA genotype. Most recently, in a case-control study from North Carolina, Shaheen et al. (16) reported an increased risk of colorectal cancer among individuals with *HFE* mutations. The increased risk, however, was only statistically significant among the African American participants (OR, 2.1; 95% CI, 1.1-3.9). Although the risk among white participants was >1, it was not statistically significant (OR, 1.2; 95% CI, 0.8-1.6). In comparing the effects of the C282Y and H63D mutant alleles, only the risk associated with the H63D mutant allele was significant (OR, 1.44; 95% CI, 1.04-1.98).

In contrast to the positive studies, several studies with null findings have been reported. Neither MacDonald et al. (17) in Australia nor van der A et al. (20) in the Netherlands found an association between the C282Y mutant allele and risk. Similarly, Altes et al. (18) in Spain found no relationship with either the C282Y mutant allele or the H63D mutant allele and risk of colorectal cancer.

In support of the null studies based on genotyping data, two studies of relatives of patients with HH also found no association between mutant alleles and colorectal neoplasia. Nelson et al. (19), using the same approach as in their study of parents of patients with HH, studied colorectal neoplasia among siblings of patients with HH. In contrast to the parent study, the sibling study reported no increased risk. A similar finding was reported by Elmberg et al. (21) in a large Swedish record-linkage study. The investigators linked the medical records of 1,847 patients with HH and 5,973 first-degree relatives to the national cancer registry and found that neither the patients with HH nor their relatives had an increased risk.

Why some studies have reported an association between *HFE* mutant alleles and colorectal neoplasia and others have not is not entirely clear. One possibility is that some studies have relied on identifying heterozygotes genetically and others have relied on identifying heterozygotes by familial relationship to an individual with hemochromatosis. Although these different approaches result in some incongruity, positive and negative findings have been reported by both approaches. Another possibility is that an association between heterozygosity and colorectal neoplasia might exist among relatives of patients with HH that does not exist among nonrelatives because the families are exposed to other environmental or genetic factors that increase iron stores enough for HH to become manifest. The parent study of Nelson et al. (3) would support such a postulate, although their sibling study (19) would not. The Swedish record linkage study also does not argue that heterozygotes in HH families are at increased risk of colorectal neoplasia.

Table 2. Risk estimates of *HFE* and *TFRC* genotypes

Genotype	Cases, *n* (%)	Controls, *n* (%)	OR	Adjusted OR*
HFE H63D†				
CC	458 (72.1)	489 (75.2)	Ref.	Ref.
CG	164 (25.8)	146 (22.5)	1.20 (0.93-1.55)	1.26 (0.97-1.64)
GG	13 (2.0)	15 (2.3)	0.93 (0.44-1.97)	0.84 (0.38-1.85)
P (trend)			0.31	0.24
HFE IVS2+4				
TT	297 (46.8)	293 (45.1)	Ref.	Ref.
TC	257 (40.5)	287 (44.2)	0.88 (0.70-1.12)	0.89 (0.70-1.13)
CC	81 (12.8)	70 (10.8)	1.14 (0.08-1.63)	1.14 (0.79-1.64)
P (trend)			0.94	0.93
HFE C282Y‡				
GG	560 (88.2)	571 (87.8)	Ref.	Ref.
GA	70 (11.0)	76 (11.7)	0.94 (0.67-1.33)	0.90 (0.63-1.28)
AA	5 (0.8)	3 (0.5)	1.70 (0.40-7.14)	1.86 (0.43-7.96)
P (trend)			0.99	0.87
TFRC G142S				
GG	122 (19.2)	130 (20.0)	Ref.	Ref.
GA	325 (51.2)	317 (48.8)	1.09 (0.81-1.46)	1.13 (0.84-1.53)
AA	188 (29.6)	203 (31.2)	0.99 (0.72-1.36)	1.02 (0.74-1.42)
P (trend)			0.83	0.99

*Adjusted for sex, age, smoking, and intakes of total iron, total fiber, total energy, and family history of colorectal cancer.
† HFE H63D allele associated with hemochromatosis = G.
‡ HFE C282Y allele associated with hemochromatosis = A.

Among the studies that have reported genotype data, neither positive study (15, 16) was unambiguously supportive of the *HFE*-colorectal neoplasia link. Beckman et al. (15) only found a risk of colorectal neoplasia when the population was stratified by *TFRC* $Ser^{142}Gly$ genotype. In addition, the study used as controls a convenience sample of individuals who may or may not have provided an appropriate comparison to the cases. In the Shaheen et al. (16) study, the relationship between *HFE* mutations and risk was statistically significant only among the Black participants. This was an unanticipated result given that the frequency of the *HFE* mutations is considerably lower among Blacks than among Whites. Also, surprisingly, the result was only significant among individuals who were heterozygous for the H63D mutation, which results in a less severe phenotype than does the C282Y mutation. Taken together, the studies that have been reported to date suggest that there is little relationship between *HFE* mutations and colorectal neoplasia. If any relationship does exist, it is likely to be in only a subset of the population.

The hypothesis that there would be an association between *HFE* mutations and colorectal neoplasia is based on the supposition that heterozygotes have higher iron stores (15, 16). In support of this supposition, several studies have

Table 3. Risk estimates of *HFE* genotype combinations stratified by *TFRC* G142S polymorphism

HFE Genotypes					*TFRC* G142S GG/GA		*TFRC* G142S AA	
H63D	IVS2+4	C282Y	OR (95% CI)	Adjusted OR (95% CI)	OR (95% CI)	Adjusted* OR (95% CI)	OR (95% CI)	Adjusted* OR (95% CI)
WW	WW	WW	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
WW	WC+CC	WW	0.83 (0.63-1.10)	0.82 (0.61-1.09)	0.73 (0.52-1.03)	0.69 (0.49-0.99)	1.10 (0.67-1.83)	1.12 (0.66-1.89)
WW	WW	WA+AA	1.01 (0.66-1.53)	1.00 (0.65-1.54)	0.93 (0.56-1.54)	0.94 (0.56-1.59)	1.20 (0.56-2.54)	1.19 (0.54-2.60)
WG+GG	WC+CC	WW	1.08 (0.81-1.43)	1.12 (0.84-1.49)	1.06 (0.76-1.49)	1.10 (0.78-1.57)	1.10 (0.66-1.84)	1.15 (0.68-1.96)
WW	WC+CC	WA+AA	0.64 (0.31-1.32)	0.61 (0.29-1.27)	0.85 (0.37-1.99)	0.83 (0.34-2.00)	0.28 (0.06-1.37)	0.25 (0.05-1.31)
WG+GG	WC+CC	WA+AA	1.32 (0.45-3.85)	1.15 (0.37-3.54)	1.12 (0.33-3.73)	1.07 (0.29-3.93)	2.25 (0.20-25.4)	1.41 (0.12-16.7)

NOTE: Abbreviations: W, wild-type allele; C, cytosine; A, adenine; G, guanine.
*Adjusted for sex, age, and smoking and intakes of iron, total fiber, total energy, and family history of colorectal cancer.

Table 4. Risk estimates of *HFE* haplotypes (H63D-IVS2+4-C282Y)

Haplotype	Cases, *n* (%)	Controls, *n* (%)	OR	Adjusted OR*
W-W-W	771 (60.7)	791 (60.8)	Ref.	Ref.
W-C-W	229 (18.0)	251 (19.3)	0.94 (0.76-1.15)	0.92 (0.75-1.14)
W-W-A	80 (6.3)	82 (6.3)	1.00 (0.72-1.38)	0.98 (0.70-1.36)
G-C-W	190 (15.0)	176 (13.5)	1.11 (0.88-1.39)	1.13 (0.89-1.42)

NOTE: Haplotype data reported in order of H63D-IVS2+4-C282Y. *n* = number of haplotypes.
Abbreviations: W, wild-type allele; C, cytosine; A, adenine; G, guanine.
*Adjusted for sex, age, smoking, and intakes of total iron, total fiber, total energy, and family history of colorectal cancer.

reported higher mean levels of iron indices among heterozygotes (32–34). It should be noted, however, that in almost all of the reports, the higher mean levels are due to a small minority of the population. For example, although Bulaj et al. (33) reported higher mean levels of serum iron, transferrin saturation, and ferritin among heterozygotes, the authors noted that only 18% of the male heterozygotes and 11% of the female heterozygotes had transferrin saturation levels more than 2 SDs above the mean of persons with no mutations. Similarly, in recently published data from White individuals in the NHANES III study, only 13.2% of C282Y heterozygotes and 9.3% of H63D heterozygotes had high (~45%) transferrin saturation levels (35). These frequencies were not significantly different from the percentage found among persons with neither mutation (8.7%). Similar findings have also been reported by Adams (36), who found that only 8.6% of heterozygotes had elevated transferrin saturation levels and only 11% had elevated ferritin levels. Even among relatives of patients with HH, only 21 to 25% have been reported to have increased serologic measures of iron (32). Thus, the great majority of heterozygotes do not have increased iron indices, so it may not be surprising that studies of *HFE* and colorectal neoplasia have reported inconsistent results.

A lack of association between *HFE* mutations and colorectal neoplasia does not preclude a relationship between iron levels and colorectal neoplasia. At least 25 studies of iron and colorectal neoplasia have been published since Graf and Eaton (37) first suggested the hypothesis that fiber might prevent colon cancer via iron chelation. Among the published studies, eight have reported a positive association between either iron intake or serologic measures of iron and colorectal neoplasia (1, 2, 4, 38-42), nine have reported an inverse association (43-51), and seven have reported no association (52-58). The great majority of the studies have been either case-control or screening studies in which serologic iron status was determined at the time of neoplasia diagnosis. Given that colorectal neoplasia may bleed prior to diagnosis, these designs are not optimal to study the relationship between iron and colorectal neoplasia. Three prospective studies have been reported thus far, with mixed results (refs. 54, 55) and NHANES I). Herrinton et al. (45) reported an inverse association between transferrin saturation and risk of colorectal cancer, which was significant only among men. Kato et al. (54), reporting data from the New York University Women's Health Study, found no overall association between colorectal cancer and serologic iron indices, except ferritin, which was significantly inversely associated with risk. They did find, however, a positive association between risk in the proximal colon and iron intake. Results of NHANES I follow-up were reported in three separate manuscripts (1, 2, 42). In the first report, colorectal cancer was significantly associated with both serum iron indices and iron intake. In the second report, only the association with iron intake remained significant. In the final report, iron intake was significantly associated with risk in the proximal colon and serum iron was associated with an increased risk among women only.

When the studies are broken down into those that studied cancer and those that studied adenoma, there is more support for an iron-cancer association than there is for an iron-adenoma association. Among the 13 studies that examined colorectal cancer, 6 reported a positive association (1, 2, 38-40), 5 reported an inverse association, and 2 reported no association (54, 57). In contrast, among the adenoma studies, two reported a positive association (4, 41), six reported an inverse association (43, 44, 47-49) and six reported no association (52, 53, 55-58). The greater number of positive iron-cancer than iron-adenoma studies may suggest that iron acts as a promoter of colorectal cancer rather than as an initiator. Alternatively, the finding of an iron-proximal cancer association in two prospective studies and little support for an iron-adenoma association may indicate that the association is predominantly with proximal disease.

The design of the current study had the advantage of ensuring that cases and controls came from the same source population and were screened with a standardized procedure (i.e., cases were not detected due to symptoms). The large study population allowed us to confine the analysis to cases with advanced adenoma, which have a higher potential for malignant transformation and are a particularly meaningful intermediate outcome for studying factors related to colorectal cancer. However, because we restricted our study to advanced adenomas, we could not examine whether *HFE* mutations were associated with any adenoma but only whether they were associated with adenomas of increased malignant potential. Similarly, we could not examine the relationship between *HFE* mutations and colorectal cancer. Because blood samples were collected from our population at the time that adenoma were diagnosed, we also could not examine prediagnostic serologic iron stores. Finally, because our population was screened using flexible sigmoidoscopy rather than colonoscopy, we could not rule out the possibility that the comparison group had a neoplasm in the proximal colon. The literature, however, suggests that the risk of proximal neoplasia among persons who have no distal neoplasia is ~2.4%, thus not representing a potential for significant bias (59).

In summary, the results from our study suggest that there is little relationship between *HFE* mutations and colorectal adenoma. Whether iron is a risk factor for colorectal neoplasia remains unclear and should be further examined in studies using a prospective design.

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